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IMMUNOCHEMICAL IDENTIFICATION OF THE 1,25-DIHYDROXYVITAMIN D_3 RECEPTOR PROTEIN IN HUMAN HEART

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A number of recent clinical observations suggest that vitamin D_3 plays an important role in maintaining normal cardiovascular function, either directly through its receptor in cardiac muscle, or indirectly through its infuence on circulating levels of calcium or on other regulatory factors. By using an antibody directed against the recombinant vitamin D_3 receptor, we have identified the receptor protein for $1,25(OH)_2D_3$ in tissue from two human hearts. The identification of the $1,25(OH)_2D_3$ receptor in human heart lends credence to the hypothesis that $1,25(OH)_2D_3$ directly effects the human heart and may be involved in several clinically relevant pathological conditions involving the vitamin D_3 endocrine system.

KEYWORDS: vitamin D; receptor; human heart.

INTRODUCTION

The receptor for 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the active metabolite of vitamin D_3 has been identified in several different tissue types, aside from the intestine and bone, including the pancreas, skin, kidney, testis, lung, and rat heart (Norman *et al.*, 1982). This receptor functions as a trans-acting transcriptional regulatory protein, similar to other nuclear hormone receptors such as the thyroid hormone receptor and the retinoid receptors. Thus, it has been shown that upon coupling with the ligand, the receptor binds as a dimer to a specific hormone response element in the promoter region of hormone-sensitive genes to regulate gene expression (Beato, 1989). The $1,25(OH)_2D_3$ receptor has been linked to the classical actions of vitamin D_3 in regulating calcium homeostasis via actions in the kidney intestine and bone (DeLuca, 1988), and the more recently discovered actions of the hormone in regulating cell growth and differentiation in several cell types (DeLuca, 1988).

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Both our own and Walters' laboratories showed that the $1,25(OH)_2D_3$ receptor exists in the rat heart (Simpson *et al.*, 1985; Walters *et al.*, 1986). Subsequently, we demonstrated that vitamin D_3 deficiency directly increases cardiac contractility and induces myocardial hypertrophy (Weishaar and Simpson, 1989), suggesting a physiological role for $1,25(OH)_2D_3$ in the regulation of heart function. We observed that the increase in heart size was due to both an increase ventricular collagen content (Weishaar and Simpson, 1989) and an increase in myocyte cell number. By using primary cultures of ventricular myocytes, we and others (Walters *et al.*, 1987; O'Connell *et al.*, 1994) were able to test the direct actions of $1,25(OH)_2D_3$ on the cardiac myocyte. Recently, we found that $1,25(OH)_2D_3$ directly decreases c-myc levels, DNA synthesis and cell proliferation. In addition, we observed that $1,25(OH)_2D_3$ blocks induced myocyte maturation as evidenced by it increasing the levels of the immature form of creatine kinase and reducing total myosin levels (manuscript in preparation).

In this report, our goal was to determine if the receptor for $1,25(OH)_2D_3$ was present in human heart tissue. This is an initial step towards relating our findings regarding the effects of vitamin D_3 deficiency on the rat heart and the direct actions of

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$1,25(OH)_2D_3$ on the cardiac myocyte, to several clinical reports on the relationship between cardiac myopathy and human vitamin D_3 deficiency. To this end, we detected the presence of the $1,25(OH)_2D_3$ receptor protein in preparations from two individual human hearts. Therefore, this report provides evidence that human heart cells possess the necessary cellular apparatus to respond to $1,25(OH)_2D_3$ and supports the possibility of a direct effect of this hormone on human heart.

MATERIALS AND METHODS

The monoclonal antibody to the recombinant human vitamin D_3 receptor was obtained from Chemicon International (Temecula, CA).

Tissue preparation

Rat $1,25(OH)_2D_3$ receptor preparations were made from the intestinal mucosa of adult female Sprague–Dawley rats. Intestinal extracts were prepared by homogenizing mucosa in 9 volumes of TKED buffer (10 mM Tris-Cl, 0.3 M KCl, 1 mM EDTA, 5 mM dithiothreitol, pH 7.2) containing 2 mM PMSF and 10 mM leupeptin using a Tissumizer (Tekmar, Cincinnati, OH). Large debris was removed by brief centrifugation at 3000 ***g*** for 5 min at 4°C. A cytosolic fraction was obtained by centrifugation of the homogenate at 100,000 ***g*** for 60 min at 4°C. Cytosolic protein concentration was determined and samples were stored at −20°C. Human heart receptor preparations were similarly made from the left ventricle of two separate hearts obtained post-mortem from donated cadavers. Heart number one was from an 86-year-old White, female patient who died of Alzheimer's dementia, heart number two was from an 80-year-old White female who died of a myocardial infarction. Tissue was obtained within 3 days of death and immediately stored at −20°C. The left ventricular tissue was minced and heart cytosol extracts were prepared as described above for intestine.

Western Blot analysis

Cytosolic protein samples from the rat intestine and the two human heart preparations were separated on a 10% polyacrylamide gel and then transferred to Immobilon paper (Millipore, Bedford, MA). The blot was blocked with buffer containing 1% bovine serum albumin (10 mM tris, 0.1% Tween-20, and 1% bovine serum albumin, pH 7.4). The blot was probed for 1.5 h with the anti-vitamin D_3 receptor antibody (Chemicon, Temecula, CA), then washed three times with blocking buffer and incubated for 1 h with a secondary antibody conjugated to horseradish peroxidase. The blot was then washed five times with Tween-TBS (10 mM tris and 0.1% Tween-20, pH 7.4). Finally, the blot was developed using enhanced chemiluminescence (Amersham, Clearbrook, IL) and exposed to X-ray film. Densitometry was performed to determine the relative abundance of the receptor.

RESULTS

We and others previously demonstrated the existence of the $1,25(OH)_2D_3$ receptor in rat heart (Simpson, 1983; Simpson *et al.*, 1985; Walters *et al.*, 1986). In this report we identify the receptor in human heart tissue. Cytosolic preparations made in TKED buffer, from both rat intestinal mucosa and left ventricular muscle from two human hearts, were analysed for the $1,25(OH)_2D_3$ receptor protein by Western Blot analysis (Fig. 1). The receptor is identified as a 55 kD protein in Fig. 1 (identified as VDR), in agreement with the findings of Allegretto and Pike (1984), and was seen in both human heart preparations. In each human heart sample, it appears that two minor bands of 54 and 56 kDa are also present, but not seen in the rat intestinal preparation. This may be explained by the fact that five times more total protein was used to blot for the receptor in the human heart. DeLuca (1988) reports the existence of three 55 kDa $1,25(OH)_2D_3$ receptor protein isoforms, as observed by 2-D gel electrophoresis. Thus, what is observed in Fig. 1 may be these three forms of the receptor. These three observed forms may represent differentially phosphorylated forms of the human receptor, as suggested by Simpson *et al.* (1983). Figure 2 shows the relative abundance of the receptor as determined by densitometry. The figure reveals that the levels of the receptor in the heart tissue is clearly less than that found in the intestine. We are not able to make any conclusion as to why there appears to be greater levels of the receptor protein in heart two *vs* heart one. However, this may relate to variations in human heart levels of the receptor. This report thus provides immunochemical evidence for the existence of $1,25(OH)_2D_3$ receptors in human heart ventricular muscle.

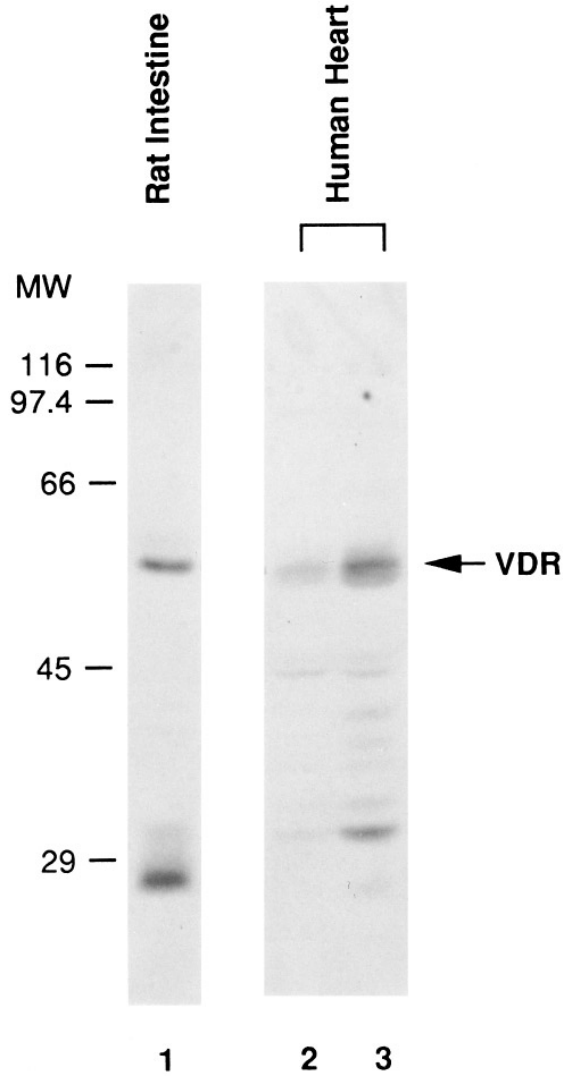

Fig. 1. Western Blot for the $1,25(OH)_2D_3$ receptor. Cytosolic protein preparations were made from rat intestinal mucosa and human heart, and $1,25(OH)_2D_3$ receptor levels were determined by Western blot analysis (see methods). Lane 1: 20 mg of rat cytosolic intestinal protein. Lane 2: 100 mg of cytosolic protein from human heart one. Lane 3: 100 mg of cytosolic protein from human heart two. The $1,25(OH)_2D_3$ receptor is marked by the symbol VDR on the right side of the figure, and molecular weight standards are shown on the left.

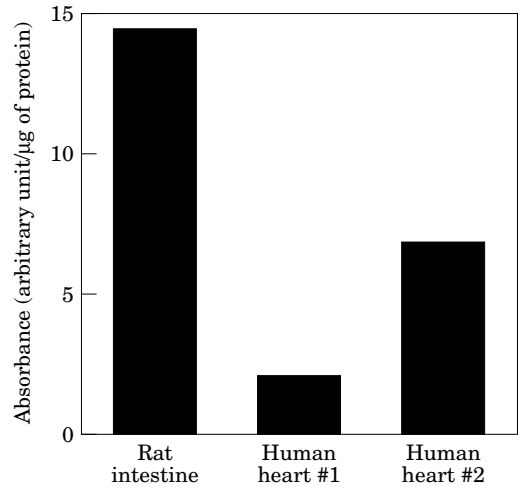

Fig. 2. Relative abundance of the $1,25(OH)_2D_3$ receptor. Densitometry was performed on the blot shown in Fig. 1 to determine the relative abundance of the $1,25(OH)_2D_3$ in the different tissue preparations. The graph presents the amount of receptor protein present in each condition in arbitrary absorbance units standardized to the amount of protein applied to each lane.

DISCUSSION

A number of observations suggest that vitamin D_3 plays an important role in maintaining normal cardiovascular function, either directly through its receptor in cardiac muscle, or indirectly through its influence on circulating levels of calcium or on other regulatory factors. Interestingly Scragg *et al.* (1990) showed that there is a correlation between reduced blood levels of 25-hydroxyvitamin D_3 and myocardial infarction in humans. Recent studies of the elderly in the Boston area showed that a significant number of the patients analysed were vitamin D_3-deficient (Webb *et al.*, 1990) and other studies have shown that there is a decrease in circulating levels of both $1,25(OH)_2D_3$ and calcium with increasing age (Fugisawa *et al.*, 1984). In addition, the possibility exists that altered levels of vitamin D_3 may play a role in the changes in cardiac function which can accompany ageing (Fugisawa *et al.*, 1984), diabetes (Nyomba *et al.*, 1985), lengthy bedrest or immobilization, and weightlessness (Nixon *et al.*, 1979; Leach *et al.*, 1983), since circulating levels of $1,25(OH)_2D_3$ are reduced by these conditions. Taken together, these studies suggest that circulating levels of vitamin D_3 and the hormone $1,25(OH)_2D_3$ have an impact on cardiovascular function with potential clinical relevance. The identification of the $1,25(OH)_2D_3$ receptor in human heart lends credence to the hypothesis that $1,25(OH)_2D_3$ directly effects the human heart and may be involved in several clinically relevant pathological conditions involving the vitamin D_3 endocrine system. Further, it provides the first step in relating our findings regarding the effects of vitamin D_3 deficiency in the rat and the direct effects of the hormone on the cardiac myocyte, to determining the actions of vitamin D_3 in a clinically relevant system.

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